Review of Clinical Pharmacology and Pharmacokinetics

INTERNATIONAL EDITION

VOLUME 27, 2013 ❁ No 2

Issue Devoted to Papers Presented at the

14th Medicinal Chemistry Conference
Drug Discovery and Design

Organized by the

Departments of Chemistry, Medicine and Pharmacy of the University of Patras, Patras, Hellas

May 9-12, 2013
Patras, Hellas

ISSN 1011-6583

Review of Clinical Pharmacology and Pharmacokinetics

INTERNATIONAL EDITION

Articles published in this Journal are ***Indexed*** or ***Abstracted*** in:

• Chemical Abstracts • Elsevier' Bibliographic Databases: Scopus, EMBASE, EMBiology, Elsevier BIOBASE • SCImago Journal and Country Rank Factor

Further informations regarding the Journal can be seen in the web address: http://pharmakonpress.gr

Review of Clinical Pharmacology and Pharmacokinetics
INTERNATIONAL EDITION

☸

Published three times a year by PHARMAKON-Press
Publisher Responsible According to the Law
Helen S. Plessa
9A Kanari str., GR-1532, Nea Penteli, Athens, Hellas
Tel.- Fax 00302109756332
Email: splessas@otenet.gr & stplessas@hotmail.com

Τετραμηνιαία Έκδοση από την ΦΑΡΜΑΚΟΝ-Τύπος
Ιδιοκτήτης-Υπεύθυνος κατά το Νόμο Ελένη Πλέσσα του Σταύρου
Κανάρη 9Α, GR-15232, Νέα Πεντέλη, Αθήνα, Ελλάς
Τηλ.- Fax 00302109756332
Email: splessas@otenet.gr & stplessas@hotmail.com

☸

☸

Original Papers, Review Articles, as well as short preliminary communications will be considered for publication and should be send to the Editors-in Chief
Πρωτότυπες Ερευνητικές Εργασίες, Άρθρα Επισκόπησης και Ενημέρωσης, καθώς και Κλινικές Μελέτες με άμεση σχέση το Φάρμακο και τη Θεραπευτική πρέπει να αποστέλλονται στους Εκδότες

☸

Online ISSN 1011-6583

Articles published in this Journal are ***Indexed*** or ***Abstracted*** in:
• Chemical Abstracts • Elsevier's Bibliographic Databases: Scopus, EMBASE, EMBiology, Elsevier BIOBASE • SCImago Journal and Country Rank Factor

VOLUME 27 ❁ 2013 No 2

CONTENTS

GENERAL INFORMATIONS

REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS INTERNATIONAL EDITION

The Journal aims to promote optimum drug therapy by providing original papers and review articles covering important aspects of clinical and applied Pharmacology and Therapeutics. The focus of the Journal comprises drug evaluation reviews, which provides a detailed focus on different properties, i.e. dosage, toxicology, drugs interactions and place in therapy of both newer and established drugs. Other Review Articles offer state-of-the-art literature surveys covering broader topics. Practical Therapeutics Articles and Leading Articles provide recommendations for specific situations of connections or emerging areas, respectively.

The Journal publishes, in special issues, papers presented at:

- the *Conferences with International Participation Medicinal Chemistry: Drug Discovery and Design organized by the Departments of Chemistry and Pharmacy of the University of Patras, Hellas*
- the *Panhellenic Congresses of Pharmacology organized by the Hellenic Society of Pharmacology*

The *scientific standard* of the papers, which are accepted for publication, is controlled by the Editorial Board or by other Experts in the various fields of Pharmacology, Pharmacokinetics and Thepapeutics.

INSTRUCTIONS TO AUTHORS

English is the preferred language for all papers. However, papers in French, German or other European languages can also be submitted, provided they are accompanied by an English summary

FORMAT: Summary, Introduction, Materials and Methods, Results, Discussion Acknowledgements and References

Manuscripts: These should mention, on the first page, the *Title*, *Author(s)* and the *Name of the Institution* at which the work was done. The complete *address* of the author, including Postal area code number, should be given under the rubric *Send reprint requests to*. Papers should follow the general form: *Introduction, Materials and Methods, Results, Discussion* and *References*. Drugs must be referred to by their generic or chemical name, but may be identified by trade name in parenthesis or o footnote. All papers should be submitted in duplicate.

Summary: A summary in English (maximum length 200 words) must accompany all manuscripts.

Key words: A list of key words should be submitted, after summary

References: These should be numbered in the paper and listed under *References* in order of their appearance in the text. The author(s) surname followed by the initials should be given first, then the complete title of the article, the name of the Journal or Magazine (abbreviated according to the Index Medicus), the volume number, page numbers and year of publication in parenthesis.

Letter from Guest Editor

Medicinal Chemistry Graduate Program
Drug Discovery and Design: A Program of Excellence

The *Medicinal Chemistry Graduate Program Drug Discovery and Design* of the University of Patras has completed its 16th year of operation. The Program, a joint collaboration of the Departments of Chemistry, Medicine and Pharmacy, has been successful with outstanding research and academic activities in the field of Medicinal Chemistry. The Program has attracted the interest of world leading scientists for participation and research collaboration. Each year a distinguished scientist is honored for his/her contribution to Biomedical Research and Science.

So far the Program has honored outstanding scientists in the field: *Ada Yonath*, Nobel in Chemistry (2013); *Kleomenis Barlos* (2012); *James D. Watson*, Nobel in Medicine and Physiology (2011); *Andrew V. Schally*, Nobel in Medicine and Physiology (2010); *Dimitrios Nanopoulos* (2009); *Jean-Marie Lehn*, Nobel in Chemistry (2008); *Kyriakos Nikolaou* (2007); *Aristidis Patrinos* (2006); *Charalambos Gavras* (2005); *Konstantinos Sekeris* (2004); *Michael Maragoudakis* (2003); *Chris Platsoukas* (2002); *Athanasios Giannis* (2001); *Vasso Apostolopoulou* (2000).

The 14th Medicinal Chemistry Conference, in co-organization with the 2nd Seedrug Workshop, was held in the Cultural and Conference Center of the University of Patras on May 13-15, 2013. The Guest of Honors were *Ada Yonath*, Professor of Biology, Nobel in Chemistry and Professor *Stamatis Krimizis*, Head of the National Council of Research and Development (ESET). The title of Professor Ada Yonath's Lecture was *From Basic Research to the New Generation Antibiotics*, and of Professor Krimizis were *Excellence and Innovation in Greek Universities and Research Institutes* and *The Odyssey of the 50-year Exploration of the Solar System*. Professor Yonath was honored by the Departments of Pharmacy and Biology of the University of Patras with the title of *Doctor Honoris Causa*.

The main research interests of the Program are focused on the Organic and Peptide Synthesis of Biomolecules, Rational Design with Aided Computer and Modeling Methods, Biological Evaluation *in vivo* and *in vitro*, Molecular Biology, Molecular Medicine, Toxicology, Biochemical Analysis, Pharmacognocy, Pharmacokinetics, Research Methods. The Program has organized, since 2000, fourteen Conferences with International Participation.

Over two hundred and fifty students have graduated so far from the Program with MSc and PhD Degrees. The Program provides highly trained students to the benefit of the European Economy and Society. Emphasis is given to applied pharmaceutical research. Innovative Products and

methods from the graduate students are published annually in high stand Journals. Graduate Research is the Key for Progress and Development in European and Worldwide dimension. The Program has been evaluated as a Centre of Excellence in Greece for its outstanding academic activities. The Program is an example of Excellence in the country, relating basic research with applied. It is the first graduate program in Greece, labeled by the title *Euromaster*, after evaluation by the ECTN Association.

The Guest Editor, on behalf of the Postgraduate Program Committee, wishes to express his deep appreciation to all contributors of this Issue. We also thank the Editorial Board of *Review of Clinical Pharmacology and Pharmacokinetics,* in particular Journal Editors Prof. S. T. Plessas and Dr C. T. Plessas for the invitation and for providing the suitable and high-stand forum through which important findings of this research will become available to the scientific community.

The Guest Editor
John M. Matsoukas
Professor of Chemistry, University of Patras, Greece
Medicinal Chemistry: Drug Discovery and Design

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 57 (1013)

From Basic Research to Advanced Antibiotics

Ada Yonath

Department of Structural Biology, Weizmann Institute, Rehovot 76100, Israel

Key words: Antibiotics, ribosomes, basic research

Ribosomes are the universal cellular apparatuses that translate the genetic code into proteins. Composed of proteins and RNA, among which the RNA moieties perform almost all functional tasks, they posses spectacular architecture accompanied by inherent mobility that facilitate their smooth and efficient performance. The stunning level of conservation of a pocket-like region containing the site for peptide bond formation hints that a remnant of a prebiotic bonding entity is functioning in the contemporary ribosomes.

Owing to their fundamental role, ribosomes are targeted by many antibiotics, each paralyzing the ribosomes by binding to a specific functional site. Their binding modes, inhibitory action and synergism pathways have been elucidated. The mechanisms leading to bacterial resistance to ribosomal antibiotics and issues concerning the ways towards combating the resistance will be discussed.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 58 (1013)

New Directions in the Immunotherapy of Multiple Sclerosis: Myelin epitopes conjugated with Mannan

John M. Matsoukas

Department of Chemistry, University of Patras, GR-265 00, Patras, Greece, Eldrug S.A. Pharmaceutical Company

Key words: Multiple sclerosis, immunotherapy, myelin epitopes, mannan, new directions

This communication highlights new directions in the research for treating Multiple Sclerosis. Therapeutic approaches for multiple sclerosis, involve the design and use of peptide analogues of disease-associated myelin epitopes to induce peripheral T cell tolerance or the use of immunotherapeutic techniques to develop Th1/Th2 responses followed by release of appropriate cytokines. Immunodominant Epitopes MBP83-99, PLP139-151, MOG35-55 of human proteins MBP, PLP, MOG of myelin sheath have been the tools in our laboratories in Patras for the Design Synthesis and Preclinical Evaluation of our rationally designed linear and cyclic analogues conjugated to mannan *via* [Lys-Gly] bridge. Specific analogues have been found to immune rats rendering them potential therapeutic vaccine drugs in the Immunotherapy of Multiple Sclerosis. Furthermore, our cyclic MBP83-99 peptides, for the first time to be reported as HLA and MHC binders and more stable compared to linear counterparts, possess a series of important immunomodulatory properties rendering them as putative drugs for treating multiple sclerosis and potentially other Th1 mediated autoimmune diseases. In the light of the results and findings in our research, the main immunodominant peptides MOG35-55, PLP139-151 and MBP83- 99 and their head to tail cyclic counterparts conjugated to reduced mannan have been selected for further investigation. Currently, we are investigating linear and cyclic analogues conjugated to mannan in oxidized (OM) or reduced (RM) forms via Lys-Gly bridge. Specific analogues in a number of preliminary vaccination and therapeutic protocols have shown interesting properties as potential vaccine drugs in the treatment of Multiple Sclerosis. A potent MOG35-55 analogue conjugated with Mannan has been selected for toxicology evaluation and development towards Clinical trials.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 59 (1013)

DNA and Graphene: A Fruitful Encounter

Theodore Christopoulos

Professor, Department of Chemistry, University of Patras, GR-265 00, Patras, Hellas

Key words: DNA, analysis, graphene

DNA analysis has found a wide spectrum of applications including pharmacogenetics, detection of mutations, detection of various pathogens, diagnosis and monitoring of disease. The progress in this field is remarkable. Indeed, for many years, these analyses required tedious and time consuming procedures based on radioactive isotopes. On the contrary, DNA biosensors are small and portable devices that enable simple, rapid and low cost DNA analysis without the need of radioactivity. Graphene is a one-atom-thick sheet of carbon atoms ordered in a two-dimensional honeycomb structure that exhibits remarkable electrical, mechanical and optical properties. Graphene oxide (GO)-based biosensors exploit the preferential interaction of GO with single-stranded DNA, over double-stranded DNA, and the fact that GO is an excellent acceptor for fluorescence resonance energy transfer. Graphene is also an excellent electrical conductor, and its use opens the door for the development of devices suitable for DNA sequencing.

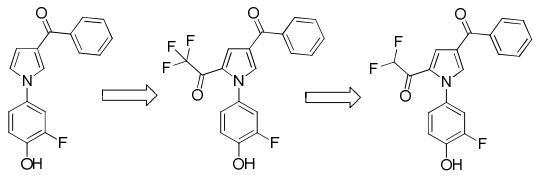
REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 60 (1013)

Towards Efficient Non-Anionic Aldose Reductase Inhibitors

Maria Chatzopoulou, Vassilis J. Demopoulos

Department of Pharmaceutical Chemistry, School of Pharmacy, *Aristotle* University of Thessaloniki, 54124 Thessalonikı, Hellas

Key words: Aldose reductase, inhibitors, non-anionic, efficient



Aldose reductase's (ALR2) involvement on the onset and progression of diabetes secondary complications has grown attention over the years, and moreover, recent evidence point towards ALR2's implication in inflammatory pathologies (1,2). As such ALR2 comprises a compelling target for medicinal chemistry. In the plethora of aldose reductase inhibitors (ARIs) synthesized so far two categories are the most studied, namely that of cyclic imides and carboxylic acid derivatives. However, a number of cyclic imide derivatives emerged with acute side effects and carboxylic acids presented with poor membrane penetration (2). In our previous work we found out that derivatives of 2-fluorophenol[3] could act as micromolar ARIs. Particularly active was a bis-substituted derivative bearing the trifluoroacetyl moiety. In this work we expanded the structure activity relationships to find out that a difluoroacetyl analogue exhibited submicromolar IC_{50} (443 nM) while retaining an optimal physicochemical profile, as indicated by molecular obesity indices such as LE and BEI. This compound could serve as a new lead for a series of ARIs that have optimum membrane permeation along with significant inhibitory activity.

REFERENCES

1. Alexiou P., Pegklidou K., Chatzopoulou M., Nicolaou I., Demopoulos V.J.: Aldose reductase enzyme and its impliction to major health problems of the 21(st) century *Curr. Med. Chem. 16*: 734-752 (2009)

2. Chatzopoulou M., Alexiou P., Kotsampasakou E., Demopoulos V.J.: Novel aldose reductase inhibitors: a patent survey (2006--present). Expert Opin. Ther. Pat. 22: 1303-1323 (2012)

3. Chatzopoulou M., Patsilinakos A., Vallianatou T., Stefek M., Ragno R., Tsantilli-Kakoulidou A., Demopoulos V.J.: *J. Med. Chem.* (Submitted). (2013)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 61 (1013)

Effects on Oxidative Status of Lenses and Brain Areas of Developmental Rats during Cataractogenesis Using Sodium Selenite and of Co-administration of Blueberry Leaves Extract

Anastasia-Varvara Ferlemi[1], Olga E. Makri[2], Marigoula Margarity[3], Constantine D. Georgakopoulos[2], Fotini N. Lamari[1]

[1]Department of Pharmacy, [2]Department of Ophthalmology, Medical School, [3]Department of Biology, University of Patras, Patras GR-26500, Hellas

Key words: Blueberry leaves extract, Na_2SeO_3, developmental rats, cataractogenesis

The goal of the present study was to examine the effects of the administration of high dosage of Na_2SeO_3, that is a rather common model for the induction of cataractogenesis via increased oxidative stress, as well as the effects of the coadministration of Na_2SeO_3/aqueous extract of blueberry leaves on the oxidative status of developmental rat lenses. Concurrently, the effects on important cerebral regions of these rats were studied for the first time. Developmental Wistar rats were randomly divided in three groups: *Se* (20 μmol Na_2SeO_3/kg subcutaneously - post natal (PN) day 10), *SeBbL* (20 μmol Na_2SeO_3/kg subcutaneously - PN day 10/100 mg dry weight of blueberry leaves extract/kg intraperitoneal - PN day 11 & 12), *control* (saline on the respective days). On the PN day 21, lenses were examined for cataract formation and cortex, midbrain and cerebellum were isolated. The activities of catalase, superoxide dismutase, glutathione peroxidase, the concentration of GSH, GSSG (GSH/GSH + GSSG) and the levels of lipid peroxidation (MDA, malonyldialdehyde) were determined in lenses and in cerebral regions. The results show that the administration of high concentration of Na_2SeO_3 induces cataractogenesis through significant changes of all the antioxidant/oxidant indices, whereas the coadministration of blueberry leaves protects the lenses from the oxidative changes. The cerebral regions respond with different pattern; the high/toxic dose of Na_2SeO_3 causes significant increase (< 20%) of lipid peroxidation in cortex and midbrain of the developmental rats, whereas the cerebellum is less vulnerable. Blueberry leaves coadministration protects the cerebral tissues from lipid peroxidation and activates differential antioxidant enzymes (i.e. SOD in cere-bellum, GPx in midbrain) indicating tissue-specific effects.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 62 (1013)

Design and Synthesis of Immunodominant MOG Epitopes of Myelin Using Microwave Synthetic Protocols, Conjugated with Mannan: Confirming of the Coupling Reaction by SDS-Page Electrophoresis

A. Tapeinou[1], K. Kyrtata[1], M. Androutsou[2], T. Tselios[1]

[1]Department of Chemistry, University of Patras, Patras GR-26500, Hellas
[2]Eldrug S.A., Pharmaceutical Company, GR-26504, Platani, Patras, Hellas

Key words: Myelin, immunodominant MOG epitopes, mannan, design, synthesis, microwave synthetic protocol, SDS-Page Electrophoresis

Multiple Sclerosis (MS) is a slowly progressive, immunologically mediated disease of the central nervous system (CNS), characterized by inflamemation and demyelination of white matter in the brain and spinal cord. MS is generally considered to be an autoimmune disease involving CD4+ and CD8+ T lymphocytes and B cells. This is strongly supported by the strong association of the MHC class II gene with disease and the ability of TH1 and Th17 CD4+ T lymphocytes to drive disease in an animal model for MS named Experimental Autoimmune Encephalomyelitis (EAE) (1,2). In this study the synthesis of MOG (Myelin Oligodendrocyte Glycoprotein), a myelin protein epitope, conjugated with the poly-saccharide mannan is reported. Mannan has successfully been used as a peptide carrier to mannose receptor of macrophages and dendritic cells. Certain experiments have shown that peptides conjugated with the oxidized form of mannan induce Th1 immune response, while the reduced form of mannan induce Th2 immune response (3,4). The aim of this study is to change the Th1/Th2 balance in patients with MS. In particular, the epitopes MOG_{35-35} RAT and MOG_{35-35} HUMAN, were synthesized and they were conjugated with the oxidized and reduced form of mannan, using the decapeptide $(Lys\text{-}Gly)_5$ as a bridge. The synthesis of myelin analo-gues was achieved using microwave peptide synthesizer (5). The purity of final products was verified by RP-HPLC and their identification was achieved by ESI-MS. Analogues conjugated to mannan in oxidized and reduced form. Conjugation reaction between peptide analogues and mannan was confirmed by SDS-PAGE electrophoresis (6).

REFERENCES

1. Spyranti Z., Dalkas G.A., Spyroulias G.A., Mantzourani E.D., Mavromoustakos T., Friligou I., Matsoukas J.M., Tselios T.V.: Putative bioactive conformations of amide linked cyclic myelin basic protein peptide analogues associated with experimental autoimmune encephalomyelitis*. J. Med. Chem. 50*: 6039-6047 (2007)
2. Katsara M., Deraos G., Tselios T. Matsoukas M.T., Friligou I., Matsoukas J., Apostolopoulos V.J.: Design and synthesis of a cyclic double mutant peptide (cyclo(87-99)[A91,A96]MBP87-99) induces altered responses in mice after conjugation to mannan: implications in the immunotherapy of multiple sclerosis*. Med. Chem. 52*: 214-218 (2009)
3. Apostolopoulos V., Barnes N., Pietersz G.A., McKenzie I.F.: *Ex vivo* targeting of the macrophage mannose receptor generates anti-tumor CTL responses. *Vaccine 18*: 3174-3184 (2000)
4. Apostolopoulos V., Pietersz G.A., Gordon S., Martinez-Pomares L., McKenzie I.F.: Aldehyde-mannan antigen complexes target the MHC class I antigen-presentation pathway. *Eur. J. Immunol. 30*: 1714-1723 (2000)
5. Friligou I., Papadimitriou E., Gatos D., Matsoukas J., Tselios T.: Microwave-assisted solid-phase peptide synthesis of the 60-110 domain of human pleiotrophin on 2-chlorotrityl resin*. Amino Acids 40*: 1431-1440 (2010)
6. Tselios, T.V., Lamari, F.N., Karathanasopoulou, I., Katsara, M., Apostolopoulos, V., Pietersz, G.A., Matsoukas, J.M., Karamanos, N.K.: Synthesis and study of the electrophoretic behavior of mannan conjugates with cyclic peptide analogue of myelin basic protein using lysine-glycine linker. *Anal. Biochem. 347*: 121-128 (2005)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 63 (1013)

Study of Collagen's Thermal Hydrolysis Using Raman Spectroscopy

Sofia Kouvaritaki[1], Panagiota Papaspyridakou[1], Sofia Panteliou[2], Christos Kontoyannis[1,3], Malvina Orkoula[1]

[1]Department of Pharmacy, [2]Department of Mechanical Engineering and Aeronautics, University of Patras, Patras, Hellas; [3]Institute of Chemical Engineering and High Temperature Chemical Processes, FORTH, GR-26500, Patras, Hellas

Key words: Collagen's thermal hydrolysis, Raman spectroscopy

Gelatin is the thermal degradation product of collagen, a highly cross-linked fibrillar protein. Raman spectroscopy was used to study chemical and conformational changes of collagen fibers associated with heating. Several parts of biogenic materials (e.g. human mandibles, human meniscus) were analyzed with micro-Raman spectroscopy. Spectra were recorded from specimens before and after immersing them in water at 45^{o}C for 24 hours. In this way, thermal hydrolysis of collagen was triggered. After deconvolution of bands under the proline-hydroxyproline area (830-910 cm^{-1}) a change in the sub-bands was observed. Proline and hydro-xylproline comprise approximately one-fourth of the amino acids in collagen. In the thermally hydrolyzed specimens the emergence of a new sub-band at ~865 cm^{-1} was observed which was attributed to gelatin. Raman spectro-scopy is a technique able to detect changes in the organic matrix of biogenic materials due to degradation conditions.

Acknowledgement: The project was financially supported by University of Patras, *K. Karatheodori Project* (C.194)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 64-65 (1013)

Crimean-Congo Hemorrhagic Fever Virus and Hantaviruses: Seroprevalence and Risk Factors in Humans in Achaea, Western Greece

Maria Sargianou[1], George Panos[1], Charalambos Gogos[1], Anna Papa[2]

[1]Division of Infectious Diseases, Department of Internal Medicine, Patras University General Hospital, Patras, Hellas
[2]Department of Microbiology, Medical School, *Aristotle* University of Thessaloniki, Thessaloniki, Hellas

Key words: Crimean-Congo hemorrhagic fever virus, hantaviruses, seroprevalence, risk factors, humans, Achaea, Western Greece

Hantaviruses can cause hemorrhagic fever with renal syndrome (HFRS) in Eurasia and Hantavirus pulmonary syndrome in the Americas. Humans acquire infection after direct contact with infected rodents or their excreta, which mostly occurs by inhaling virus-contaminated aerosol of their excreta (1). In Greece, the predominant strain is Dobrava-Belgrade and 1-5 clinical cases are being diagnosed annually. After the recent diagnosis of a hantavirus case in Achaea (2), the present seroepidemiological study was designed in order to determine the hantavirus seroprevalence in the local population and to assess possible factors playing a role in acquisition of this viral infection.

Crimean-Congo hemorrhagic fever virus (CCHFV) cause a hemorrhagic syndrome and patients present with fever and non-specific symptoms, which often progress to a serious hemorrhagic syndrome, with average case fatality rate being up to 30% (3). CCHFV is transmitted to humans through the bite or crushing of infected ticks, and through close contact with the blood or tissues of viremic patients or livestock (4). The significant presence of human CCHFV antibodies among the Greek population, as reported in previous studies (5), is contradictory to the low number of diagnosed CCHF cases (only one clinical case in Rhodope (6). Since this prefecture has not been previously studied for CCHFV, the aim of the present study was to estimate the seroprevalence in humans in an attempt to detect possible endemic foci of the virus in a region where the local climate, along with the local geomorphology and vegetation, potentially favors the survival and spread of Hyalomma spp. ticks, the most common vector of the virus, and to assess possible risk factors for the acquisition of CCHFV infection.

Between March and July 2012, 207 serum samples were prospectively collected from all 5 municipalities of Achaea. The participants were randomly selected among persons without signs of infectious disease, referred for routine blood testing or for blood donation to Patras University General Hospital and to 4 additional primary healthcare centers (in Chalandritsa, Kalavryta, Kato Achaea and Klitoria). Information regarding the virus and the purpose of the present study was provided by physicians, and written consent was obtained from all participants before sampling. A standard questionnaire regarding demographics and potential risk factors for the two viruses was then completed.

Serum samples were tested for hantaviruses IgG antibodies and CCHFV IgG antibodies by ELISA (Anti-Hanta Virus Pool 1 *Eurasia* ELISA IgG, Euroimmun, Lübeck, Germany and Vector-

Best, Koltsovo, Novosibirsk, Russia, respectively), according to manufacturer instructions. All the ELISA-positive samples were tested for CCHFV IgM antibodies by the same commercial ELISA. In addition, the ELISA-positive serum samples were further tested by indirect immunofluorescent assay (IFA) in order to eliminate possible cross-reactions (and to discriminate between the CCHFV nucleoprotein-specific (N) and the CCHFV glycoprotein Gc-specific (GPC) antibodies). The data were statistically analyzed using SPSS 20.0 (IBM, Chicago, Illinois, USA) and univariate logistic regression analysis was performed to calculate the odds ratio (OR) and the 95% confidence intervals (95% CI), and to identify possible risk factors for the two infections. In total, 20/207 (9.66%) tested serum samples tested hantaviruses IgG positive, with seropositivity rates differing in the municipalities of Achaea; in the municipality of West Achaea, a local seroprevalence maximum was encountered, where 5/27 tested samples were found positive. Univariate analysis showed that: age (OR 1.036, 95%CI 1.011-1.062), peridomestic sighting of rodents (in <200m around the place of residence, OR 4.538, 95% CI 1.019-20.219) and ownership of a storing shed (OR 2.892, 95%CI 1.004-8.327) were statistically significant risk factors for acquisition of hantavirus infection.

As for CCHFV, 7/207 (3.4%) serum samples tested CCHFV IgG positive. Seropositive individuals were encountered in rural areas in 4 of the 5 municipalities, most of whom lived in Erymanthos, a municipality in central Achaea, where 4/27 tested samples were positive (15%). Univariate analysis showed that: increased age (OR 1.056, 95% CI 1.009-1.104, p=0.018), agropastoral occupation (OR 6.792, 95% CI 1.450-31.813, p=0.015), contact with sheep (p<0.001) and goats (OR 8.190, 95% CI 1.740-38.563, p=0.008), former tick bite (OR 9.128, 95% CI 1.711-48.691, p=0.010), living at an altitude of ≥400m (OR 17.500, 95% CI 3.221-95.080, p=0.001), living on a specific land type (on a non-irrigated arable land, OR 24.25, 95% CI 1.807-325.389, p = 0.016, and on a land principally occupied by agriculture, with significant areas of natural vegetation, OR 24.25, 95% CI 3.399-173.023, p=0.001) and living in the Erymanthos municipality (OR 10.261, 95% CI 2.159-48.767, p=0.003) were significantly associated with CCHFV seropositivity in Achaea. A statistically significant negative association of altitude <80m of place of residence with CCHFV seropositivity (OR 0.059, 95% CI 0.007-0.498, p=0.009) was also found.

In conclusion, the current study presents a relatively high CCHFV IgG seroprevalence and an unexpectedly high hantavirus seroprevalence in Achaea prefecture, indicating the circulation of the two viruses in the area, and identifies several environmental, social and geomorphologic predisposing factors to these infections. Studies in ticks and in rodents are needed to identify the circulating CCHFV and hantavirus strains in Greece respectively, which could lead to a better understanding of the ecology and epidemiology of these viruses in the country. Clinicians should include CCHFV and hantavirus infections in the differential diagnosis of acute febrile cases accompanied by hemorrhagic manifestations and/or renal impairment, especially when the patients have an agro-pastoral occupation.

REFERENCES

1. Jonsson C.B., Figueiredo L.T., Vapalahti O.: A global perspective on hantavirus ecology, epidemiology, and disease. *Clin. Microbiol. Rev. 23*: 412-41 (2010)
2. Sargianou M., Watson D.C., Kampiotis D., Papa A., Economopoulou A., Gogos C., Panos G.: Febrile disease in a Bulgarian emigrant with acute renal failure, thrombocytopenia, bilateral subconjunctival haemorrhage and hypoxemia. *J. Clin. Virol. 54(1)*: 2-5 (2012)
3. Papa A.: Crimean-Congo hemorrhagic fever and hantavirus infections. In: (H.C. Maltezou, A. Gikas, eds) Tropical and emerging infectious diseases. Pp. 49-73, Research Signpost, Kerala (India), 2010
4. Yilmaz G.R., Buzgan T., Irmak H., Safran A., Uzun R., Cevik M.A., Torunoglu M.A.: The epidemiology of Crimean-Congo hemorrhagic fever in Turkey, 2002-2007. *Int. J. Infect. Dis. 13*: 380-386 (2009)
5. Sidira P., Maltezou H.C., Haidich A.B., Papa A.: et al., Seroepidemiological study of Crimean-Congo haemorrhagic fever in Greece, 2009-2010. *Clin. Microbiol. Infect. 18(2)*: E16-19 (2012)
6. Papa A., Dalla V., Papadimitriou E., Kartalis G.N., Antoniadis A.: Emergence of Crimean-Congo haemorrhagic fever in Greece. *Clin. Microbiol. Infect. 16*: 843-847 (2010)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 66 (1013)

Combining Cinnamate and Paracetamol in a Hybrid Molecule with Antiinflammatory and Analgecic Activity: A Pharmacochemical Study

Aikaterini Peperidou[1], Dimitra Hadjipavlou-Litina[1], Christos Kontogiorgis[1], Vasilia Kalamara-Tsambika[2] and Dorothea Kapoukranidou[2]

[1]Department of Pharmaceutical Chemistry, School of Pharmacy, [2]Department of Physiology, Medical School, *Aristotle* University, Thessaloniki GR-54124, Hellas

Key words: Cinnamate, paracetamol, combining, hybrid molecule, antiinflammatory, analgecic activity

Hybrid drugs combine two drugs in a single molecule with the goal of creating a chemical entity more medically effective than its individual components. Today, there is an increased interest in the combination of two pharmaco-phores on the same scaffold. Continuing our research in the design of antioxidant/anti-inflammatory agents based on the *α, β* unsaturated acids scaffold we synthesized a hybrid molecule by the combination of a cinnamic acid which has been found to act as a potential inhibitor of LOX and paracetamol, a non steroidal well known analgesic drug (1,2). It is widely known that cinnamic acid derivatives are interesting from the point of biological activities and present particular synthetic interest.

The compound has been identified using IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. Lipophilicity as Rm values was determined using RPTLC. The hybrid molecule has been screened for its biological activity *in vitro* on: a) soybean lipoxygenase inhibition, b) interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, c) inhibition of lipid peroxidation and in vivo e) for the antiinflammatory and analgesic activity and f) on nerve regeneration and functional recovery following peripheral nerve injury using a rat sciatic nerve crush model.

Our results indicate that this hybrid molecule presents potent antioxidant, antiinflammatory activity, accelerates functional recovery following sciatic nerve crush in the rat and it appears to be a promising agent for treating peripheral nerve injuries.

Acknowledgments: A. Peperidou and D. Hadjipavlou-Litina acknowledged the Research Committee of *Aristotle* University for financial support. This research is a part of a project financed by the Research Committee of Aristotle University of Thessaloniki.

REFERENCES

1. Pontiki E., Hadjipavlou-Litina D., Litinas K., Nicolotti O., Carotti A.: Design, synthesis and pharmacobiological evaluation of novel acrylic acid derivatives acting as lipoxygenase and cyclooxygenase-1 inhibitors with antioxidant and anti-inflammatory activities. *Eur. J. Med. Chem. 46*: 191-200 (2011)
2. Smith H.S.: Potential analgesic mechanisms of acetaminophen. *Pain Physician 12*: 269-280 (2009)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 67 (1013)

Chemical Mapping of Human Meniscus Using micro-RAMAN Spectroscopy

Panagiota Papaspyridakou[1], Dionysios Papachristou[2], Panagiotis Megas[3], Christos Kontoyannis[1,4] and Malvina Orkoula[1]

[1]Department of Pharmacy, University of Patras; [2]Department of Anatomy-Histology-Embryology, School of Medicine, University of Patras and Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA; [3]Department of Orthopedic Surgery, School of Medicine, University of Patras; [4]ICE/HT-FORTH, Patras, Hellas

Key words: Human meniscus, human, chemical mapping, micro-Raman spectroscopy

Meniscus is a fibrocartilagenous formation located in the knee joint. Osteoarthritis is a disease caused by degeneration of the articular cartilage. In the present study, micro-Raman spectroscopy was employed in order to study the chemical changes induced on human meniscus due to osteoarthritis. Collagen type II in healthy condition was found to gradually give place to type I, which, in badly affected parts, prevailed. Different types of glycosaminoglycans (GAGs) were also traced. In healthy areas, chondroitin sulfate was solely identified, while in the osteoarthritic meniscus dermatan sulfate and heparan sulfate were predominant. Mineral crystals (apatite and calcite) were also spotted in certain cases in the meniscus rims.

Acknowledgement: The project is financially supported by University of Patras, *K. Karatheodori* Project (C.194).

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 68 (1013)

Study of Disinfection of Water of Swimming Tanks Using Alternative Technologies in Pilot Model

D. Papadopoulou, A. Birmpa, A. Kokkinos and A. Vantarakis

Environmental Microbiology Unit, Lab of Public Health, Medical School, University of Patras, Patras, Hellas

Key words: Swimming water tanks, disinfection, alternative technologies, pilot model

Swimming pool water disinfection is essential in order to minimize the risk of microbiological hazards and to protect swimmers from infection. Traditionally, chlorine is used to disinfect swimming pools. However, it produces residual toxic compounds such as trihalomethanes, haloacetic acids, which are toxic for the bather's health. Two alternative, promising and environmentally friendly disinfection methods are the use of UV light and ultrasound. Using UV and US for disinfection, lower the cost and chemical usage, lower disinfection by-products and provide for less intensive maintenance.

The aim of this study was to access the effect of the use of UV light and US for disinfection of different microorganisms using a laboratory swimming pool model which simulated an actual swimming pool of Olympics' dimensions. The bacterial strains that were used were *Escherichia Coli* NCTC 9001, *Enterobacter Aerogenes* NCTC 10006, *Staphylococus Aureus* NCTC 6571, *Pseudomonas Aeruginosa* NCTC 10662 and Enterococcus faecalis NCTC 775. *Ps. Aeruginosa* is an opportunistic bacterium which can accumulate in biofilms and it is very common cause of infection problems in swimming pools. *S. aureus* has been used as it is a pathogen causing several health problems. *E. Coli* and *E. Aerogenes* have been used as they are indicators of water quality. The reduction of all the microorganisms was effective and reached up to 1-3 logs after 6 hours of continuing UV disinfection apart from *Ps. Aeruginosa* which didn't show significant reduction.

REFERENCES

1. Ponyakapo M., Soontornchai S., Paopuree P.: Cancer risk assessment from exposure to trihalomethanes in tap water and swimming pool water. *J. Environ. Sci. (China) 20*: 372-378 (2008)
2. Florentin A., Hautemanière A., Hartemann P.: Health effects of disinfection by-products in chlorinated swimming pools. *Int. J. Hyg Environ. Health 214*: 461-469 (2011)
3. Dyck R., Sadiq R., Rodriguez M.J., Simard S., Tardif R.: Trihalomethane exposures in indoor swimming pools: a level III fugacity model. *Water Res.* **45***:* 5084-5098 (2011)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 69 (1013)

Development and Validation of a Sensitive and Reliable HPLC-UV Method for the Determination of Busulfan in Human Plasma to be used for Dose Individualization

Efthymios Neroutsos[1], E. Archontaki[2], A. Dokoumetzidis[1], G. Valsami[1]

[1]Laboratory of Biopharmaceutics & Pharmacokinetics, Faculty of Pharmacy and [2]Laboratory of Analytical Chemistry, Department of Chemistry, National & Kapodistrian University of Athens, Athens, Hellas

Key words: Busulfan, human plasma, determination, sensitive & reliable HPLC-UV method

Busulfan is widely used as an alternative to total body irradiation (TBI), in the preparative regimens before hematopoietic stem cell transplantation (HSCT). At 1983 Santos (1) used oral Bu at 1 mg/kg every six hour for 16 doses in combination with cyclophosphamide (Cy) as a preparative pattern, for patient with acute muelogenous leukemia (AML) who were undergoing HSCT. Busulfan charachterized by big variability. Furthermore, Anderson et al. (2) observed that *i.v* busulfan in 0.8/kg every 6 hours for 16 doses is safer than oral Bu. Moreover, Rusell et all. (3) optimized the i.v. administration of Bu at 3.2mg/kg once daily for four consecutive days that proved to be the safest. However, Bu presents an enormous variability and therefore its administration must be individualized. The aim of the present work was to develop and validate a simple and sensitive RP-HPLC method with UV detection for the determination of Busulfan in patient's plasma, ideal for pharmacokinetic studies.

1,6-bis-(methanesulfoxul)hexane was used as internal standard (IS). Both Busulfan and IS are characterized by poor UV absorption. Therefore, they were derivatized by sodium diethyldithiocarbamate (DDTC). Sample preparation process involved the addition of 20 μl of IS to 250 μl of control or patient plasma and vortexed for 5 s. then busulfan was extracted with 2 ml of ethyl acetate. After centrifugation for 5 min at 645 g, the supernatant was placed in clear class tubes and 120 μl of DDTC were added. The mixture was vortexed for 5 s, dried at 60 ^{0}C under air stream and dissolved in 200 μl of methanol. Finally, 20 μl were injected into the HPLC system. An isicratic elution program was applied on a Hypersil BDS RP-C_{18} column (150×4.6 mm, 5 μm) attached to a BDS RP-C_{18} precolumn (10×4.6 mm, 5 μm). The mobile phase consisted of methanol and water (80:20 v/v) at a flow rate of 1.0 ml/min. Detection occurred at 251 nm. Finally, validation of the developed method was performed on specificity, linearity range, accuracy, recovery, precision, stability and limits of detection and quantification.

REFERENCES

1. Santos G.W., Tutschka P.J., Brookmeyer R., et al.: Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. *N. Engl. J. Med. 309*: 1347-1353 (1983)
2. Andersson B.S., Kashyap A., Gian V., et al.: Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: a phase II study. *Biol. Blood Marrow Transplant. 8:* 145-154 (2002)
3. Russell J.A., Tran H.T., Quinlan D., et al.: Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. *Biol. Blood Marrow Transplant. 8*: 468-477 (2002)

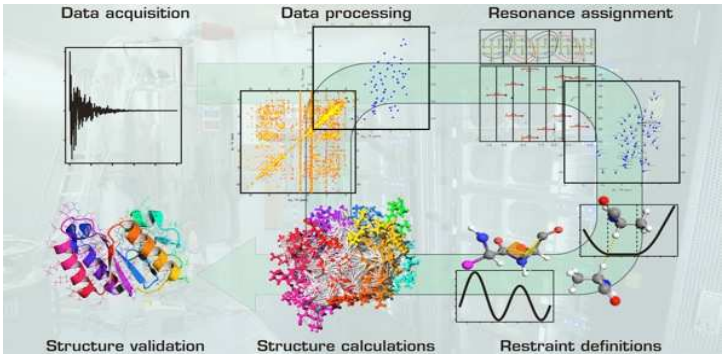
REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 70 (1013)

Analysis and Assignment of 3D Heteronuclear NMR Spectra of the Dictyostelium Discoideum Lupus Antigen Protein N-RRM Domain

A. Myxaki[1], M. Apostolidi[2], D.J. Vourtsis[1], C.T. Chasapis[1], C. Stathopoulos[2], D. Bentrop[3], G.A. Spyroulias[1]

[1]Departments of Pharmacy & [2]School of Medicine, University of Patras, GR-26504, Patras, Hellas; [3]Institute of Physiology II, University of Freiburg, Freiburg 79104, Germany

Key words: Lupus antigen (La) protein N-RRM domain, dictyostelium discoideum, 3D heteronuclear NMR spectra



The Lupus Antigen (La) protein is involved in different stages of RNA metabolism. At first, it was described as an autoantigen in patients with rheumatic diseases of Systemic lupus erythematosus or with Sjogren's syndrome. The La protein contains a La motif (LAM) in the N-terminal region followed by the RRM (RNA Recognition Motif) and an RRM in the C-terminal region.

The present study, presents the NMR study of the N-terminal domain of the La protein from the microorganism Dictyostelium discoideum, which comprises the LAM along with the NRRM domain (a motif that is known to act as a RNA Recognition Motif). NMR study is based on the 2D and 3D homo- and hetero-nuclear experiments. Through the analysis of these data, the resonances of the 1H/13C/15N nuclei of each individual residue were identified kai the data extracted were sued for the prediction of the secondary structure elements, using the software TALOS (1) and PECAN (2), both available through internet. According to the analysis performed, the LAM exhibit a high content of α-helical con-formation, while 2 or 3 polypeptide segments were predicted to adopt a β-strand conformation. On the other hand the NRRM, exhibits 2 major and one smaller α-helical segment and 4 poly-peptide segments bearing β-strand conformation.

REFERENCES

1.TALOS: (http://spin.niddk.nih.gov/NMRPipe/talos/)
2. PECAN: (http://pine.nmrfam.wisc.edu/PECAN/)

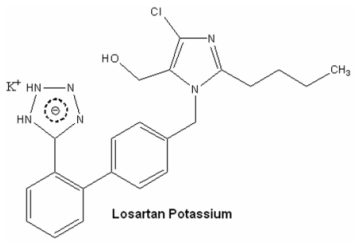
REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 71 (1013)

Transdermal Delivery of AT1 Angiotensin II Receptor Antagonists

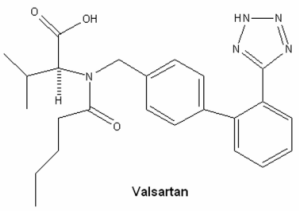
Michaila Michalatou[1], Maria-Eleni Androutsou[1,3], Theodore Tselios[1], Pigi Katsougraki[1], George Agelis[1,3], Demitrios Vlahakos[2] and John Matsoukas[1,3]

[1]Department of Chemistry, University of Patras, GR-26500, Rio, Patras, Hellas; [2]Department of Internal Medicine, *Attikon* University Hospital, GR-12462, Athens, Hellas; [3]Eldrug S.A., Pharmaceutical Company, GR-26504, Platani, Patras, Hellas

Key words: Angiotensin II, AT1 receptor antagonists, transdermal delivery



Losartan Potassium



Valsartan

The Renin-Angiotensin System (RAS) plays a determinant role in blood pressure regulation and in the pathogenesis of hypertension. Losartan and Valsartan are two well known orally active AT1 antagonists (Sartans). Transdermal drug delivery systems (TDDS) are an alterative therapeutic approach and offer pharmacological advantages compared to the oral route.

Our objective was to investigate the *in vitro* transdermal delivery of Losartan and Valsartan in human skin using franz cells and several formulations with a variety of enhancers (CPEs). Furthermore, the antihypertensive response of Wistar rats was recorded after 3, 6, 8 and 24h of transdermal administration. The results showed satisfactory drug release and antihypertensive activity even after 24h of administration. In conclusion, transdermal administration of Sartans may be feasible and appears to be a modern alternative route for prolonged activity in antihypertensive therapy.

Acknowledgments: This research has been co-financed by the European Union (European Social Fund, ESF) and Greek national funds through the Operational Program *Education and Lifelong Learning* of the National Strategic Re-ference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

REFERENCES

1. Nishida N., Taniyama K., Sawabe T., Manome Y.: Development and evaluation of a monolithic drug-in-adhesive patch for valsartan. *Int. J. Pharm. 402*: 103-109 (2010)
2. Samad A., Ullah Z., Alam M.I., Wais M., Shabaz M., Shams S.: Transdermal drug delivery system: patent reviews. *Recent. Pat. Drug Del. Formul. 3:* 143-152 (2009)
3. Wokovich A.M., Prodduturi S., Doub W.H., Hussain A.S.; Buhse L.F.: Transdermal drug delivery system (TDDS) adhesion as a critical safety, efficacy and quality attribute. *Eur. J. Pham. Biopham. 64:* 1-8 (2006)
4. Ahad A., Aqil M., Kohli K., Sultana Y., Mujeeb M., Asgar A.: Role of novel terpenes in transcutaneous permeation of valsartan: effectiveness and mechanism of action. *Drug Dev. Ind. Phar. 37:* 583-596 (2011)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 72 (1013)

Design, Synthesis and Solution Conformational Studies of New Analogues of χ-Conopeptide MrIA

V. Mertziani[1], D. Tsigrimani[2], C. Potamitis[2], P. Cordopatis[1], M. Zervou[2*], V. Magafa[1*]

[1]Laboratory of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, Rio GR-26500, Patras, Hellas
[2]Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Hellas

Key words: χ-Conopeptide MrIA, analogs, design, synthesis, conformational studies

χ-Conopeptide MrIA is a 13-residue peptide (NGVCCGYKLCHOC) isolated from the venom of marine snail *Conus Marmoreus* bearing two disulfide bonds (C^4-C^{13} and C^5-C^{10}) χ-MrIA has been found to inhibit the norepinephrine transporter (NET) with potential applications in the treatment of pain (1). The amidated χ-MrIA (MrIA-NH_2) has slightly higher affinity at NET compared to the native peptide, adopting a ribbon-like fold with a β-hairpin linked by a flexible turn between G^6 and L^9 (2). Alanine scan has indicated the residues of the turn region as well as H^{11} to be critical for binding to NET (1). Moreover, N-terminal truncation affects the conformational stability by disrupting V^3-O^{12} hydrogen bonding (2). SAR studies revealed that the replacement of the N^1 with pyroglutamic acid [pGlu] could lead to improved chemical stability and increased efficacy and potency rendering Xen2174 ([$pGlu^1$]MrIA-NH_2) as a lead compound toward the selective NET inhibition (3). Inspired by the importance of the N-terminal residues, we proceeded to the synthesis of the χ-MrIA-NH_2 and its analogues comprising modifications at positions 1 and 3. The analogues contain N or pGlu or Sar (N-methyl-alanine) in position 1 while position 3 is altered to Gly(*t*Bu) (L-α-*t*-butyl-glycine) or 1-Nal (1-L-naphtylalanine). The analogues were synthesized by the Fmoc/*t*Bu solid phase methodology (4) utilizing Sieber Amide resin as solid support to provide the peptidic amide. The formation of disulfide bonds was achieved in two steps by oxidation with DMSO/H_2O (2:8, v/v) and Iodine/AcOH. NMR spectroscopy was applied for the sequential assignment and the structure elucidation of the analogues in DMSO. Experimental NOE data were further imposed as distance constraints to MD simulations. The aqueous solution NMR structure of MrIA-NH_2 (2) was used as an initial template for the MD studies. The obtained results are intended to drive a rational optimization of this class of inhibitors.

REFERENCES

1. Sharpe *I.A.,* Palant E., Schroeder C.I., Kaye D.M., Adams D.J., Alewood P.F., Lewis R.J.: Inhibition of the norepinephrine transporter by the venom peptide chi-MrIA. Site of action, Na^+ dependence, and structure-activity relationship. *J. Biol. Chem. 278:* 40317-40323 (2003)
2. Nilsson K.P., Lovelace E.S., Caesar C.E., Tynngård N., Alewood P.F., Johansson H.M., Sharpe I.A., Lewis R.J., Daly N.L., Craik D.J.: Solution structure of chi-conopeptide MrIA, a modulator of the human norepinephrine transporter: *Biopolymers 80:* 815-823 (2005)
3. Brust A., Palant E., Croker D.E., Colless B., Drinkwater R., Patterson B., Schroeder C.I., Wilson D., Nielsen C.K., Smith M.T., Alewood D., Alewood P.F., Lewis R.J.: chi-Conopeptide pharmacophore development: toward a novel class of norepinephrine transporter inhibitor (Xen2174) for pain. *J. Med. Chem. 52:* 6991-7002 (2009)
4. Fields B.G. Noble L.R.: Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int. J. Pept. Prot Res. 35:* 161-214 (1990)

Acknowledgments: The authors thank the University of Patras for the K. Karatheodoris Research Grant (to V. Magafa_2010_D.156). Part of this work was implemented under the FP7 Regional Potential project Advancement of Research Capability for the Development of New Functional Compounds (ARCADE).

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 73 (1013)

Design, Synthesis and Biological Evaluation of 5(4H)-Oxazolones

Evangelos Mavridis* and Dimitra Hadjipavlou-Litina

Department of Pharmaceutical Chemistry, School of Pharmacy, *Aristotle* University, GR-54124 Thessaloniki, Hellas. hadjipav@pharm.auth.gr, emavridis@pharm.auth.gr

Key words: 5(4H)-Oxazolones, design, synthesis, biological evaluation

5(4*H*)-oxazolones are heterocyclic compounds having a remarkable number of biological applications antimicrobial, anticancer, anti-inflammatory, analgesic (1), antidiabetic (2) and antityrisinase and have been demonstrated to be very versatile building blocks in organic synthesis, as they contain numerous reactive sites allowing for a diverse set of possible modifications. Their reactivity makes them excellent substrates for their use in diverse oriented synthesis. Using computer aided drug design and previous biological data from known 5(4H)-oxazolones we designed a series of new derivatives as possible inhibitors of lipoxygenase with antioxidant, anticancer and anti-inflammatory activities in vitro. The general procedure for the preparation of 4-(arylidene)-2-(phenyl)-5(4H)-oxazolones includes the condensation of aromatic aldehydes and hippuric acid with a stoichiometric amount of fused sodium acetate in the presence of acetic anhydrides as the dehydrating agent, a reaction which is known as the Erlenmeyer Plöchl reaction (3). The structures of the synthesized compounds were confirmed spectroscopically and by elemental analysis. The compounds were tested *in vitro* for their ability to: a) inhibit lipid peroxidation of linoleic acid, b) inhibit *in vitro* soybean lipoxygenase and c) in vivo for the inhibition of carrageenin induced rat paw edema. The results were characterized based on the structural characteristics and physicochemical properties of the molecules.

Acknowledgements: Biobyte Corp., 201 West 4th St, Suite 204, Claremont CA 91711, USA

REFERENCES

1. Apostol T.-V, Draghici C., Dinu M., Barbuceanu S.-F., Socea L.-I., Saramet I.: *Rev. Chim. (Bucharest) 62(2)*: (2011)
2. Mariappan G., Saha B.P., Datta S., Kumar D., Haldar P.K.: *J. Chem. Sci. 123*: 335-341 (2011)
3. Patil S.G., Bagul R.R., Kamble V.M., Navale V.A.: *J. Chem. Pharm. Res. 3(4):* 285-290 285 (2011)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 74 (1013)

Investigating the Amorphous Formation of Active Pharmaceutical Ingredients through Complexing with Resins Using XRD, DSC, IR and Raman Spectroscopy

Georgia Manika[1], and Christos Kontoyannis[1,2]

[1]Department of Pharmacy, University of Patras, Rio, Patras, Hellas
[2]ICE/HT-FORTH, Patras, Hellas

Key words: Active pharmaceutical ingredients, amorphous formation, resins complexing, XRD, DSC, IR, Raman spectoscopy

The amorphous phase of active pharmaceutical ingredients (APIs) is thermodynamically unstable and as a result transformation to crystal phases takes place during formulation or during the storage period. In cases when it is necessary for the API to remain amorphous e.g. when patents are involved, complexing the APIs with resins is chosen. Verification of the amorphous complex formation is an interesting analytical problem. The APIs Ivabradine HCl and Rasagiline Tartrate were complexed with a resin (Amberlite IRP88). The successful complexation was tested using XRD, DSC, IR and Raman spectroscopy. The main goal was to check if the amorphous formation was indeed a complex of the resin with the APIs or if the API became amorphous during the complexation attempt but without *bonding* with the resin. Mixtures of resin and API's (amorphous and crystalline) were prepared and compared against the complex forms by studying the thermal behavior of the mixtures and the complexes (DSC) and by examining the respective IR (ATR) and Raman spectra.
Acknowledgement: The project is financially supported by University of Patras, *K. Karatheodori* Research Program

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 75 (1013)

Levels of Linezolid in Pleural Fluid - Exudates

Dimosthenis Lykouras[1], Vasiliki Apollonatou[1], Theodore Tselios[3], Maria-Eleni Androutsou[3], Achilleas Giannopoulos[1], Apostolos Voulgaridis[1], Nikolaos Mparampatis[1], Panagiwtis Kitrou[2], Fotios Sampsonas[1], John Mastsoukas[3], Kiriakos Karkoulias[1], George Panayiotakopoulos[4] and Kostas Spiropoulos[1]

[1]Pulmonary Medicine Department, [2]Laboratory of Radiology, [4]Laboratory of Pharmacology, Department of Medicine; [3]Department of Chemistry, University of Patras, Rio GR-26500, Patras, Hellas

Key words: Linezolid, levels, pleural fluid exudates

Lung diseases such as pneumonia are in need of antibiotics. In this case, the administrated antibiotic should have good bioavailability in the lung parenchyma, in order to cure the disease successfully. The aim of this study is to examine the bioavailability of linezolid in pleural fluid, in order to reveal its importance in pleural effusions.

We included 7 patients, who were diagnosed with a pleural effusion as a result of pneumonia (parapneumonic effusion). The patients did not suffer from any chronic illness and did not have cancer of any type. We performed aspiration of the pleural effusion. Moreover, an arterial catheter was placed in order to take the blood samples in particular time points (0, 1.5, 2.5, 4, 6, 9, 12 hours after the first administration of linezolid IV) (1). At the same time points pleural fluid was also taken by a thoracic drainage tube which was placed for appropriate treatment. The specimens were centrifuged immediately and both serum and pleural fluid were stored in the freezer (-80 ^{o}C). Linezolid levels are measured with HPLC-MS technique (2). Analysis showed that these effusions are exudative pleural effusions.

The specimens were used for pharmacokinetics study of linezolid in pleural fluid of patients with parapneumonic effusion, process which has not been described in literature so far.

REFERENCES

1. Myrianthefs P., Markantonis S.L., Vlachos K., Anagnostaki M., Boutzouka E., Panidis D., Baltopoulos G.: Serum and cerebrospinal fluid concentrations of linezolid in neurosurgical patients. *Antimicrob. Agents Chemother. 50*: 3971-3976 (2006)

2. Vardakas K.Z., Kioumis I., Falagas M.E.: Association of pharmacokinetic and pharmacodynamic aspects of linezolid with infection outcome. *Curr. Drug Metab. 10(1):* 2-12 (2009)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 76 (1013)

Design, Synthesis and Biological Evaluation of Enonic Compounds

Thalia Liargkova and Dimitra Hadjipavlou-Litina

Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University, GR-54124, Thessaloniki, Hellas, hadjipav@pharm.auth.gr, thalialiargkova@yahoo.gr

Key words: Enonic compounds, design, synthesis, biological evaluation

The enonic group consists the basic structural part of a big number of biologically active molecules such as flavonoids and their derivatives. Flavonoids display a wide variety of biological activities including anti-inflammatory, antioxidant, antibacterial, anticancer, antiangiogenic, antimalarial and antileishmanial activities. The same biological activities were reported for other enone derivatives, such as chalcones and bis-substituted chalcone ethers (1,4,5).

Using computer aided drug design and previous biological data from known chalcones and flavonoid derivatives we designed a series of chalcones and bis-substituted chalcone ethers with possible inhibition on lipoxygenase, anticancer and anti-inflammatory activities in vivo (2,3,5).

Hydroxy-chalcones were synthesized via the Claisen-Schmidt condensation reaction between 4′-hydroxy-acetophenones and appropriately substituted aromatic aldehydes in basic conditions. 4'-hydroxy-acetophenone were subjected to etherification and rendered to bis-substituted chalcone ethers via the Claisen-Schmidt condensation with several aldehydes resulted (1,3,4). The structures of the synthesized compounds were confirmed by spectroscopy and elemental analysis.

The compounds were tested *in vitro* for their ability to: a) scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical in different concentrations, b) inhibit lipid peroxidation of linoleic acid, c) inhibit *in vitro* soybean lipoxygenase, d) interact with glutathione and e) inhibit in vitro ACHE.

The results were characterized based on the structural characteristics and physicochemical properties of the molecules.

REFERENCES

1. Detsi A., Majdalani M., Kontogiorgis C.A., Hadjipavlou-Litina D., Kefalas P.: Natural and synthetic 2'-hydroxychalcones and aurones: synthesis, characterization and evaluation of the antioxidant and soybean lipoxygenase inhibitory activity. *Bioorg. Med. Chem. Lett. 17*: 8073-8085 (2009)
2. Shi H.B., Zhang S.J., Ge Q.F., Guo D.W., Cai C.M., Hua W.X.: Synthesis and anticancer evaluation of thiazolylchalcones. *Bioorg. Med. Chem. Lett. 20:* 6555-6559 (2010)
3. Bandgar B.P., Gawande S.S.: Synthesis and biological screening of a combinatorial library of beta-chlorovinyl chalcones as anticancer, anti-inflammatory and antimicrobial agents. *Bioorg. Med. Chem. Lett. 18*: 2060-2065 (2010)
4. Sodani R.S., Choudhary P.C., Sharma H.O., Verma B.L.: *E-Journal of Chemistry 7*: 763-769 (2010)
5. Uriarte-Pueyo I., Calvo M.I.: Flavonoids as acetylcholinesterase inhibitors. *Curr. Med. Chem. 18*: 5289-5303 (2011)

Acknowledgement: Biobyte Corp., 201 West 4th St, Suite 204, Claremont CA 91711, USA

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 77 (1013)

SAR-analysis of Chloramphenicol dimers

Ourania N. Kostopoulou[1], George E. Magoulas[2], Dionysios Papaioannou[2] and Dimitrios L. Kalpaxis[1]

[1]Laboratory of Biochemistry, School of Medicine and [2]Laboratory of Synthetic Organic Chemistry, Department of Chemistry, University of Patras, GR-26504,Rio, Patras, Hellas

Key words: Chloramphenicol dimers, SAR-analysis

Chloramphenicol (CAM) is a broad spectrum antibiotic. It resembles the 3'-end of aminoacyl-tRNAs and inhibits protein synthesis by targeting the peptidyltransferase center (PTC). Recent crystallographic studies in bacteria *Deinococcus radiodurans*, *Escherichia coli*, and *Thermus thermophilus* showed that CAM binds into the hydrophobic crevice of PTC (1). Nevertheless, a relative study in *Haloarcula marismortui* indicated that CAM binds at the entrance to the peptide exit tunnel (2), while crosslinking experiments suggested that a similar binding site may be present in *E. coli*. (3).

To investigate the validity of these data by another approach, we decided to study CAM dimers, hoping that they could bind to both sites and exhibit superior activity. *In silico* analysis indicated that the imino groups of two molecules of CAM, when bound to the aforementioned sites, are separated by a space of 9.8 Å, corresponding to 6-7 successive single carbon bonds. Therefore, we synthesized two dimers, mag234 and mag240, in which the CAM molecules were separated via a diacyl linker of 6 carbon atoms, differing in the flexibilty of the linker. The inhibitory impact of these analogs on peptide-bond formation was kinetically measured, using a cell-free system derived from *E. coli*. Kinetics showed that the inhibitory activity of mag234 (K_i^*=1.9 μM) fell behind those of mag240 (K_i^*=0.3 μM) and the parent compound (K_i^*=0.9 μM). Beyond the importance of our findings in the mapping of the PTC, our kinetic results underscore the pivotal role of the linker properties in designing CAM dimers with improved activity.

REFERENCES

1. Schlünzen F., Zarivach R., Harms J., Bashan A., Tocilj A., Albrecht R., Yonath A., Franceschi F.: Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature* 413: 814-821 (2001)
2. Hansen J.L., Ippolito J.A., Ban N., Nissen P., Moore P.B., Steitz T.A.: The structures of four macrolide antibiotics bound to the large ribosomal subunit. *Mol. Cell*. *10*: 117-128 (2002)
3. Long K.S., Porse B.T.: A conserved chloramphenicol binding site at the entrance to the ribosomal peptide exit tunnel. *Nucleic Acids Res*. *31*: 7208-7215 (2003)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 78 (1013)

Design and Synthesis of Acrylic Acid Derivatives

Markella Konstantinidou, Christos Kontogiorgis and Dimitra Hadjipavlou-Litina

School of Pharmacy, Aristotle University, Thessaloniki GR-54124, Hellas

Key words: Acrylic acid derivatives, design, synthesis

Compounds bearing the acrylic acid moiety have been previously reported to possess promising anti-inflammatory and antioxidant activities. Lipoic acid is a naturally occurring compound that acts as an efficient antioxidant both *in vitro* and *in vivo*. Due to the fact that hybrid compounds, which combine two different pharmacophores, in some cases show an enhanced biological activity, we performed a modeling study on a number of acrylic acid derivatives and their lipoic acid adducts in order to evaluate the effect of this modification on the biological activity and to support our design.

As a biological-target, we have chosen interleukin-6 (IL-6), a pleiotropic cytokine that regulates multiple biological responses including the development of the nervous and hemato-poietic systems, acute-phase responses in inflammation and immune responses (1).

Acrylic acids were prepared by Knoevenagel condensation (2). The acids were esterified and then the aminoamides were undertaken in good yields with reflux in toluene. The hybrids were synthesized by the aminoamides and the activated N-hydroxysuccinimidyl-ester of lipoic acid (3,4). The compounds have been identified by melting points, IR, ^{1}H-NMR, ^{13}C-NMR specta and elemental analysis. The results were discussed in terms of the structural and physio-chemical properties of the compounds.

Acknowledgments: M. Konstantinidou and D. Hadjipavlou-Litina acknowledged the Research Committee of *Aristotle* University for financial support. This research is a part of a project financed by the Research Committee of *Aristotle* University of Thessaloniki.

REFERENCES

1. Hirano, T.: Interleukin 6 and its receptor: ten years later. *Int. Rev. Immunol. 16:* 249-284 (1998)
2. Pontiki, E., Hadjipavlou-Litina, D., Litinas, K., Nicolotti, O., Carotti, A.: Design, synthesis and pharmacobiological evaluation of novel acrylic acid derivatives acting as lipoxygenase and cyclooxygenase-1 inhibitors with antioxidant and anti-inflammatory activities. *Eur. J. Med. Chem. 46:* 191-200 (2011)
3. Melagraki, G., Afantitis, A., Igglessi-Markopoulou, O., Detsi, A., Koufaki, M., Kontogiorgis, C., Hadjipavlou-Litina D.J.: Synthesis and evaluation of the antioxidant and anti-inflammatory activity of novel coumarin-3-aminoamides and their alpha-lipoic acid adducts. *Eur. J. Med. Chem. 44:* 3020-3026 (2009)
4. Detsi A., Bouloumpasi D., Prousis K.C., Koufaki M., Athanasellis G., Melagraki G., Afantitis, A., Igglessi-Markopoulou O., Kontogiorgis C., Hadjipavlou-Litina D.J.: Design and synthesis of novel quinolinone-3-aminoamides and their alpha-lipoic acid adducts as antioxidant and anti-inflammatory agents. *J. Med. Chem. 50:* 2450-2458 (2007)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 79 (1013)

In Silico Screening for New Inhibitors of Human Cytosolic Phospholipase $cPLA_2$ and Molecular Docking Experiments

E. Koulouridi, V. Magrioti, G. Kokotos, T. Mavromoustakos

Laboratory of Organic Chemistry, Chemistry Department, University of Athens, Panepistimiopolis, Zografou, GR-157 71, Athens, Hellas

Key words: Cytosolic phospholipase $cPLA_2$ human, new inhibitors, *in silico* screening, molecular docking experiments

The fundamental function of enzyme $cPLA_2$, is torelease arachidonic acid from the phospholipid membranes. Arachidonic acid is the precursor for the formation of lipid mediators of inflammation, including eicosanoids. Therefore, the enzyme is an interesting target for biochemical and structural studies which can lead to a deeper knowledge of the treatment of inflammation.

A pharmacophore model has been created based on the ligand-based methodology and the process of *pharmacophore-based Virtual Screening* (VS) has been applied to commercially available compound libraries. For *hits* compounds that emerged from this process, molecular docking experiments have been performed in order to investigate their docking scoring.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 80 (1013)

In Silico Studies of 3-aryl-Coumarins as MAO-B Receptor Inhibitors

C. Koukoulitsa[1], C. Kontogiorgis[2], D. Hadjipavlou-Litina[2], T. Mavromoustakos[1]

[1]Department of Chemistry, University of Athens, Panepistimiopolis, Zographou GR-15771 Athens; [2]Department of Pharmaceutical Chemistry, School of Pharmacy, *Aristotle* University, GR-54124 Thessaloniki, Hellas

Key words: 3-aryl-Coumarins, MAO-B receptor inhibitors, *in silico* studies

Monoamine oxidase B (MAO-B) is an enzyme responsible for catalyzing the oxidative deamination of neurotransmitters such as dopamine, serotonin, and adrenaline. MAO-B selective inhibitors represent en effective treatment for relieving symptoms resulting from the loss of dopaminergic neurons in Parkinson's disease.

In an effort to discover new, more potent, and less toxic MAO-B selective inhibitors, the coumarin nucleus has emerged as a promising scaffold for MAO inhibitors. Thus, the investigation of *in silico* MAO-B inhibitory activity of twelve 3-arylcoumarin analogues has been undertaken.

Initially, the BBB (Blood Brain Barrier) penetration and the Caco-2 permeability of these compounds were predicted using the VolSurf program. Moreover, docking calculations with the Glide docking algorithm were conducted. The results of these *in silico* studies provided a promising pharmacokinetic profile and valuable insights into the enzyme-inhibitor binding interactions.

Acknowledgments: This work was supported by the Program HRAKLEITOS II.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 81 (1013)

Synthesis and 2D NMR Studies of a *β*-Cyclodextrin/LHRH Analogue Conjugate

Golfo Kordopati[1], Theodore Tselios[1], Thomas Mavromoustakos[2], Simona Golic Grdadolnik[3], Gerasimos Tsivgoulis[1]

[1]Department of Chemistry, Univetrsity of Patras, Rio, Patras, Hellas; [2]Department of Chemistry, Kapodistrian Univetrsity of Athens, Panepistimiopolis, Zographou GR-15771 Athens, Hellas; [3]Laboratory of Biomolecular Structure, National Institute of Chemistry, Ljubljana, Slovenia

Key words: *β*-Cyclodextrin/LHRH analogue conjugate, synthesis, 2D NMR studies

Cyclodextrins (CDs) are a family of cyclic oligosaccharides containing of α-D-glucose units via α-1,4-linked glucosidic bonds. They are known for their ability to encapsulate into their hydrophobic cavity *via* host/guest complexation a variety of compounds (1). Luteinizing-hormone-releasing hormone (LHRH) is a linear decapeptide which is produced in the hypothalamus under the control of neurotransmitter type compounds (2) and it is the central regulator of the reproductive system. LHRH agonist analogues are widely used for the treatment of hormone depended cancer (3). However, their amino acid skeleton is sensitive to proteolysis and sensitivity is enhanced further by the fact that LHRH analogues have low intestinal absorption and bioavailability. Aiming the reduction of the proteolysis sensitivity, we carried out the synthesis of a cyclodextrin/LHRH analogue conjugate. The 3D structure of this conjugate was further studied via 2D NMR spectroscopy and the intramolecular interactions between the peptide and the cyclodextrin moiety were explored. As it was found, the cyclodextrin cavity encapsulates intramolecularly certain parts of the peptide (side chains of amino acids).

REFERECES

1.Wenz G.: Cyclodextrine als Bausteine supramolekularer Strukturen und Funktionseinheiten. *Angew. Chem. 106*: 851-870 (1994)\

2. Lincoln D.W.: Gonadotropin-releasing hormone (GnRH): basic physiology. In: (DeGroot L.J., et al., eds) Endrocrinology. Pp. 142-151, PA: W.B. Saunders, Co., Philadelphia, 1997

3. Flanagan C.A., Millar R.P., Illing N.: Advances in understanding gonadotrophin-releasing hormone receptor: structure and ligand interactions. *Rev. Reprod. 2*: 113-120 (1997)

Acknowledgment: G. Kordopati is financial supported by BioNMR project

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 82 (1013)

Method Development for Isolation and Analysis of the Components of the *Hypericum perforatum* Oil

Nikos Kontaxis[1], Dimitrios Anagnostopoulos[3], Nikolaos-Stauros Koulakiotis[1,2], Christina Tesseromati[3], Antonis Tsarbopoulos[2,3], Fotini Lamari[1]

[1]Department of Pharmacy, University of Patras, Rio GR-26504, Patras, [2]Bioanalytical Department, Goulandris Natural History Museum, Kifisia GR-14563, Athens, [3]Department of Pharmacology, School of Medicine, University of Athens, GR-11527, Athens, Hellas

Key words: *Hypericum perforatum* oil components, isolation, analysis

Hypericum perforatum is an indigenous plant of Greece with worldwide distribution. From antiquity to today, the water-alcohol extracts of the herba are used to treat mental disorders such as anxiety and depression, whereas the *Hypericum* oily extract, for the healing of wounds, burns and ulcers. The oil is obtained by extraction of the herb within edible oil for weeks, thus imparting a deep red color. As there is no analytical method for the simultaneous determination of all main components of the oil, the aim of our work was to develop a new and simple method for quantitative isolation and simultaneous quantification of the predominant components of the oil. The isolation is carried out by adsorption chromatography on silica. Small amount of oil is added to adsorption material and eluted with chloroform to remove lipids and other lipophilic components, and then the components, that have been adsorbed on silica, are eluted with a solvent mixture of methanol/water (1% acetic acid), 9/1 v/v. The eluate is concentrated to dryness and redissolved in a small volume of solvent to be analyzed. Analysis of oil components was performed by reversed phase liquid chromatography and mass spectrometry (HPLC-DAD and LC-MS). Main components identified are phenolic acids (chlorogenic acid), flavonoids (quercetin, I3 II8-biapigenin) and the naphthodianthrone hypericin. The developed analytical method is suitable for application in quality control of Hypericum oil formulations commercially available.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 83 (1013)

Exploring the Molecular Basis of interactions of Candesartan Cilexetil (TCV-116) in Lipid Bilayers Using ^{1}H and ^{13}C CP/MAS Solid State NMR Spectroscopy

P. Kolokotroni[1], D. Ntountaniotis[2], J. Baldus[3], C. Glaubitz[3], T. Mavromoustakos[2]

[1]Department of Chemistry, University of Patras, Patras GR-26500; [2]Chemistry Department, National and Kapodistrian University of Athens, Panepistimioupolis Zographou GR-15771, Hellas
[3]Institute of Biophysical Chemistry Goethe University Frankfurt, Max von Laue Str. 9, 60438 Frankfurt, Germany

Key words: Candesartan cilexetil (TCV-116), interactions molecular basis, ^{1}H and ^{13}C CP/MAS Solid State NMR Spectroscopy

Angiotensin II receptor blockers is the newest class of approved antihypertensive agents. Candesartan cilexetil (TCV-116) is an angiotensin II receptor antagonist and a known prodrug against high blood pressure (hypertension). In this study, solid state NMR experiments were applied to explore the molecular basis of interactions of TCV-116 with lipid bilayers of dioleoyl phosphatidylcholine (DOPC) in the absence and presence of cholesterol. Cholesterol is an essential component of cellular plasma membranes in higher organisms. It interacts with membrane phospholipids and influences their physicochemical properties. The important membrane properties that are directly or indirectly influenced by membrane levels of cholesterol include solute permeability in bilayer membranes and phospholipid acyl chain mobility. The aim of this research work is to study the interactions of TCV-116 with lipid bilayers in the fluid phase in the absence and presence of cholesterol. The results will be compared with those obtained with other AT_1 antagonists.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 84 (1013)

Primed T Cells with Low Dose of Human Recombinant Interleukin-2 (hrIL2) Display Reduced Alloproliferative Capacity *in vitro*

Dimitra Kokkinou[1], Georgia Oikonomopoulou[1], Maria Liga[1], Evi Traintafyllou[1], John A. Barrett[2], Alexandros Spyridonidis[1]

[1]Bone Marrow Transplantation Unit and Laboratory, University Hospital Patras, Rio, GR-26500, Patras, Hellas; [2]National Institutes of Health, Washington DC, USA

We have recently shown that prophylactic donor lymphocyte infusions (pDLI) in patients with high risk leukemia augments the graft-versus-leukemia effect (GvL), however, they were associated with a relatively high incidence of GvHD. Strategies to preserve a beneficial GvL effect without GvHD are needed. IL2 plays dual role in immune responses, contributing to both the generation of effector T cells and the maintenance of regulatory T cells (Tregs). Recently, low dose IL2 therapy has been advanced as a potential immune modulator able to *tweak* the immune response to aid transplant tolerance and to suppress GvHD. In the present study we aimed to investigate the impact of priming lymphocytes with IL2 on their proliferative responses to allostimulation *in vitro*. Our results indicate that *in vitro* priming (p) of T cells with low (LD) and high (HD) doses of IL-2 reduces their alloproliferative capacity. However, LD p-T cells unlike HD p-T cells, displayed significant higher percentages of Tregs and a no-exhausted phenotype. In contrast to HD p-T cells, LD p-T cells presented phenotypic characteristics predictive of high capacity to persist, expand and function after adoptive cell transfer *in vivo*. *In vitro* priming of T-cells with LD-IL-2 might prove an easy, cheap and GMP compatible strategy to minimize pDLI-GvHD.

REFERENCES

1. Liga M., Triantafyllou E., Tiniakou M., Lambropoulou P., Karakantza M., Zoumbos N.C., Spyridonidis A.: High alloreactivity of low dose prophylactic donor lymphocyte infusions in acute leukemia patients transplanted with alemtuzumab. *Biol. Blood Marrow Transplant. 19:* 75-81 (2013)
2. Koreth J., Matsuoka K., Kim H.T., McDonough S.M., Bindra B., Alyea E.P. 3rd, Armand P., Cutler C., Ho V.T., Treister N.S., Bienfang D.C., Prasad S., Tzachanis D., Joyce R.M., Avigan D.E., Antin J.H., Ritz J., Soiffer R.J.: Interleukin-2 and regulatory T cells in graft-versus-host disease, *N. Engl. J. Med. 365:* 2055-2066 (2011)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 85 (1013)

Identification of Novel Selective MAGL Inhibitors Using Pharmacophore Models and Molecular Docking

T. Kellici, V. Magrioti, G. Kokotos, T. Mavromoustakos

Laboratory of Organic Chemistry, Chemistry Department, National Kapodistrian University of Athens, Panepistioupolis Zografou, GR-15771, Athens, Hellas

Key words: MAGL inhibitors selective, identification, pharmacophore models, molecular docking

The endocannabinoid system is involved in diverse processes such as control of appetite, perception of pain, inflammation, neurodegenerative diseases, anxiety, cancer and cancer related symptoms. The serine hydrolase monoacylglycerol lipase (MAGL) plays an important role linking the endocannabinoid and eicosanoid pathways together, by hydrolyzing and degrading 2-arachidonoylglycerol (2-AG), an important precursor for the synthesis of pro-inflammatory eicosanoids and by supplying free fatty acids for the production of protumorigenic signaling lipids. As a result *drugable* selective inhibitors of MAGL are attractive pharmaceutical compounds. Based on recent studies in which new potent MAGL inhibitors have been developed (mainly benzotriazol-1-yl carboxamides and O-hexafluoroisopropyl carbamates) (1,2), we have generated a pharmacophore model using LigandScout. The selected compounds have been subjected to a second selection using the docking program Glide to prioritize the hits. The compounds with favored calculated fit to both pharmacophore and protein structures were retained. The predicted binding modes were assessed by superimposition back onto the 3D pharmacophore. The promising compounds will be evaluated biologically in order to justify the pharmacophore model.

REFERENCES

1. Chang J.W, Niphakis M.J., Lum K.M., Cognetta A.B., Wang C., Matthews M.L., Niessen S., Buczynski M.W., Parsons L.H., Cravatt B.F.: Highly selective inhibitors of monoacylglycerol lipase bearing a reactive group that is bioisosteric with endocannabinoid substrates. *Chem. Biol. 19*: 579-588 (2012)

2. Morera L., Labar G., Ortar G., Lambert D.M.: Development and characterization of endocannabinoid hydrolases FAAH and MAGL inhibitors bearing a benzotriazol-1-yl carboxamide scaffold. *Bioorg. Med. Chem. 20*: 6260-6275 (2012)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 86 (1013)

Study of GAG-Collagen Interactions Using Micro-Raman Spectroscopy

Eleni Kamilari[1], Christos Kontoyannis[1,2], Fotini Lamari[1] and Malvina Orkoula[1]

[1]Department of Pharmacy, University of Patras, GR-26500 Rio, Patras, Hellas
[2]ICE/HT-FORTH, Patras, Hellas

Key words: GAG-Collagen interactions, micro-Raman spectroscopy

Collagen, the most abundant protein in mammalians tissues, is the major component of the extracellular matrix (ECM). In the ECM are also found proteoglycans consisted of glycosaminoglycan (GAG) chains covalently linked to a protein core. The GAG chains are anionic linear polysaccharides containing repeating disaccharide units of hexosamine and uronic acid. Both collagen and GAGs regulate a large number of cellular functions and many pathological conditions have been linked to GAGs. In the present work, micro-Raman spectroscopy was employed for the exploration of possible interactions between different GAGs and different types of collagen. Raman spectra of various mixtures of GAGs with collagen were acquired. Vibrational frequencies of the functional groups of both molecules were identified. The spectra of the GAG-collagen mixtures showed prominent features in the regions 800-890 cm^{-1} (collagen prolixne-hydroxyproline envelope), 920-980 cm^{-1} and 1000-1150 cm^{-1} (sulfate group envelope). A stronger *chemical preference* of collagen type I to heparin and collagen type II to chondroitin sulfate was observed.

Acknowledgement: The project is financially supported by University of Patras, *K. Karatheodori* Project (C.194)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 87-88 (1013)

In silico Studies of the Inhibition Properties of GK241 and its Analogues against Secretory Phospholipase $sPLA_2$ GIIA

S. Vasilakaki, C. Koukoulitsa, E. Barbagianni, T. Mavromoustakos, G. Kokotos

Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimioupolis, Zografou GR-157 71, Athens, Hellas

Key words: GK241, analogues, inhibition properties, secretory phospholipase $sPLA_2$ GIIA, *in silico* studies

Inflammation on the arterial wall plays an important role in all stages of atherosclerosis leading to diseases such as coronary artery disease, peripheral arterial disease and carotid atherosclerosis (1,2). Although, the exact pathway has yet to be clarified, the secretory phospholipase $sPLA_2$ GIIA promotes inflammation contributing to arachidonic acid metabolism. It is only recently that $sPLA_2$ GIIA has been identified as a bifunctional enzyme which can act either via catalysis-dependent mechanism producing arachidonic acid or indirectly contributing in the intracellular production of eicosanoids (3). The selective inhibition of group IIA towards the rest secretory phospholipase subgroups (I, III, V, IX, X, XI, XII, XIII and XIV) is of a great interest as a pharmacological target against inflammatory disease (4).
Kokotos and co-workers have developed a novel class of inhibitors based on long chain 2-oxoamide derivatives of α-amino acids (Figure 1). Most recently, *in vitro* experiments of *GK241* inhibitor (Figure 2) revealed improved IC_{50} record comparing to past results of other 2-oxoamide derivatives. Molecular Dynamics calculations (Amber) further improve our understanding of the key interactions between the *GK241* inhibitor and the binding site of the enzyme while theoretical calculations of its metabolic properties are carried out using the MetaSite software. In addition, *in silico* fragment-based drug design (5) procedure was followed for 40 compounds retrieved from the database DrugBank.ca allowing us to investigate new possible interactions between subfolders of the active site and these compounds. The above *in silico* calculations may lead us to modify the GK241 structure and potentially achieve a better binding motif.

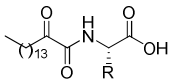


Figure 1. 2-oxoamides derivatives based on (S)-amino acids. R=side chain of the corresponding amino acid.

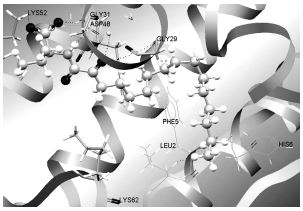


Figure 2. The binding mode of GK241 in the $sPLA_2$ GIIA active site calculated using GOLD 5.1.

Acknowledgmends: This research has been co-financed by the European Union (European Social Fund-ESF) and Greek national funds through Education and Lifelong Learning of (NSRF) - Research Funding Program: Heracleitus II

REFERENCES

1. Van Diepena, J. A.; Berbéea, J. F.P.; Havekesa, L. M.; Rensen, P. C.N. *Atherosclerosis* 2013 *in press*

2. Jashari F., Ibrahimi P., Nicoll R., Bajraktari G., Wester P., Henein M.Y.: Coronary and carotid atherosclerosis: similarities and differences. *Atherosclerosis 227*: 193-200 (2013)
3. Lee L.K., Bryant K.J., Bouveret R., Lei P., Duff A.P., Harrop S.J., Huang E.P., Harvey R.P., Gelb M.H., Gray P.P., Curmi P.M., Cunningham A.M., Church W.B., Scott K.F.: Selective inhibition of human group IIA-secreted phosphorlipase A2 (hGIIA) signaling reveals arachidonic acid metabolism is associated with colocalization of hGIIA to vimentin in rheumatoid synoviocytes. *J. Biol. Chem. 2013 in press*
4. Magrioti V., Kokotos G.: Phospholipase A2 inhibitors for the treatment of inflammatory diseases: a patent review (2010--present). *Expert. Opin. Ther. Pat. 23:* 333-344 (2013)
5. Mortier J., Rakers C., Frederick R., Wolber G.: Computational tools for *in silico* fragment-based drug design. *Curr. Top. Med. Chem. 12:* 1935-1943 (2012)

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 27: 89 (1013)

A Novel Methodology for the Quantification of Carbonyls as an Indicator of Protein Peroxidation in Organisms

V. Argiropoulou and C. Georgiou

Laboratory of Biochemistry, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, Rio GR-26500, Patras, Hellas

Key words: Carbonyls, quantification, protein peroxidation, organisms, methodology

Oxidative stress is a situation when the cells are characterized by an overcome of the antioxidant defense by oxidative attacks, which leads to the appearance of free radicals, that cause damages to the bio (macro) molecules of the cell (lipids, nucleic acids, proteins). Oxidative stress is involved in the mechanisms of both physiological (differentiation, aging) and pathological conditions (neurodegenerative diseases). Our research concerns the development of two novel methodologies for the quantification of carbonyl groups in proteins. Carbonyl groups are created in the amino acids of proteins, following the exposure of cells/organisms under high oxidative stress conditions. Therefore, protein carbonyls and especially their precise quantification is an important indicator of oxidative damage in organisms. The existing photometric method, based on the reaction of carbonyl groups with the reagent 2,4,-dinitrophenylhydrazine (DNPH) presents several disadvantages. The main disadvantages are: (1) the non-photometric variation of the resulting hydrazone from the free DNPH reagent, coupled with its isolation via protein precipitation and insufficient washing of the protein precipitate to remove non-specifically bound DNPH to the protein, and (2) the hydrolysis of the hydrazone bond at acidic pH. Therefore, the typical biochemical DNPH-based-method as well as another similar method based on the fluorescent hydrazine reagent, fluorescein 5-thiosemicar-bazide (FTC), cannot be used accurately. The novel fluorimetric method proposed overcomes the disadvantages of the classical method and gives us a more reliable methodology. The assay is based on a new reagent, Rhodamine B Hydrazine (RBH), which overcomes all the aforementioned disadvantages of the previous methods and further results in higher sensitivity (detection of less amount of protein). Thus, the accurate quantification of protein carbonyl groups with RBH is now possible in a short time and gives us an estimate of the oxidative damage in any biological sample. The practical importance of the results of this study is its application to a variety of biological tissues (mussel and mouse organs, human blood and plasma, plant tissues) as an indicator of oxidative stress assessment.